Randomized Trial of STD Control for Maternal-Infant Health, Rakai, Uganda.

Ronald H. Gray, Fred Wabwire-Mangen, Godfrey Kigozi, Nelson K. Sewankambo, David Serwadda, Fred Makumbi, Chuanjun Li, Lawrence H Moulton, Thomas C Quinn, Michael Z. Chen, Tom Lutalo, Merlin Robb, Fred Nalugoda, Noah Kiwanuka, Mary P Meehan, Katherine L O'Brien, Charlotte Gaydos, Lynn Paxton and Maria J Wawer for the Rakai Project Group*.

Affiliations

Department of Population and Family Health Sciences (RH Gray, C Li, MZ Chen), **Department of International Health** (LH Moulton, KL O'Brien), **School of Hygiene and Public Health,** and **Department of Medicine** (C. Gaydos, TC Quinn), **Johns Hopkins University, Baltimore USA**. **Institute of Public Health** (F Wabwire-Mangen, David Serwadda), **Department of Medicine and Clinical Epidemiology Unit** (NK Sewankambo), **Makerere University, Kampala, Uganda. Rakai Project, Uganda Virus Research Institute, Entebbe, Uganda** (G Kigozi, T Lutalo, F Makumbi, F Nalugoda, N. Kiwanuka). **Center for Population and Family Health, Columbia University, Joseph L Mailman School of Public Health, New York, USA** (MP Meehan, MJ Wawer). **Walter Reed Army Institute of Research, Rockville, USA** (M Robb). **National Institute of Allergy and Infectious Diseases, National Institutes of Health, Washington, DC** (TQ), **Centers for Disease Control, Atlanta, USA** (LA Paxton).

*** Rakai Project Group, Rakai Project, Uganda Virus Research Institute, Entebbe, Uganda**. Muhammad M Kiddugavu, Makko Musagara, Dativa Mukasa, Sarah Kalibbala and others)**.**

Address for correspondence

Ronald H Gray

Suite 4030

Johns Hopkins University

School of Hygiene and Public Health

615 N. Wolfe Street

Baltimore MD 21205

Phone (410) 955-7818

Fax (410) 614-7386

E-mail rgray@jhsph.edu

Acknowledgments

The study was supported by grants RO1 AI34826 and RO1 AI3426S, National Institute of Allergy and Infectious Diseases, grant 5P30HD06826, National Institute of Child Health and Development, and grant 5D43TW00010 from the Fogarty Foundation, US National Institutes of Health. Rockefeller Foundation, grant 5024-30 John Snow Inc., Pfizer Inc., and the World Bank STI Project, Uganda.

We thank Dr. S. Sempala (Uganda Virus Research Institute/Uganda Ministry of Health), Drs. R Hoff and P. Hitchcock (National Institutes of Health), Dr. M G. Fowler (Centers for Disease Control) and Dr. S. Fiscus (University of North Carolina) for their scientific contributions to this study. We also wish to thank the members of the Data Safety and Monitoring Board for their oversight, and all study participants in Rakai District Uganda, for their contributions and support.

Abstract

Background A community randomized trial assessed the effects of STD control during pregnancy on STDs, mother-to-child HIV transmission, maternal HIV acquisition and birth outcomes.

Methods, Five community clusters were randomized to an intervention arm (mass, oral antibiotic treatment for STDs), and 5 to a control arm (single dose iron/folate, referral for symptomatic STDs and syphilis), in rural Rakai district, Uganda. Pregnant women were treated once during pregnancy at varying gestations, and followed postpartum. Maternal data collected in the home, included sociodemographic/behavioral and medical history, and STD/HIV diagnoses. Infant data included anthropometry/gestational age, gonorrheal/chlamydial ophthalmia and HIV infection. Intent-to-treat analyses used multivariate cluster-adjusted rate ratios (RR), and 95% confidence intervals (95%CI), of event rates in the intervention relative to the control arm.

Results 2,070 pregnant women were enrolled in the intervention arm and 1,963 in the control arm, of whom 1,958 (94.6%) and 1,819 (92.7%), respectively, were followed up postpartum. At follow up, maternal HIV and syphilis prevalence were comparable, but rates of other STDs were significantly reduced in the intervention arm: *T. vaginalis* RR = 0.28 (95%CI 0.18-0.49), bacterial vaginosis RR = 0.78 (95%CI 0.69-0.87), *N. gonorrhoeae/C. trachomatis* RR=0.43 (95%CI 0.27-0.68), and infant ophthalmia RR=0.37 (95%CI 0.20-0.70). No effect was observed on maternal HIV acquisition or mother-to-child HIV transmission. Rates of pregnancy loss and maternal deaths were comparable between arms. Early neonatal deaths were lower in the intervention arm (RR= 0.83, 95%CI 0.71-0.97), as were proxy measures of low birth weight based on chest circumference (RR=0.68, 95%CI 0.53-0.86), combined head and chest circumference (RR = 0.76, 95%CI 0.59-0.99), and preterm delivery (RR=0.77, 95%CI 0.56-1.05).

Conclusions Control of maternal STDs during pregnancy reduced the rates of STD infections, but did not affect maternal HIV acquisition or maternal-to-infant HIV transmission. Reduction of STDs was associated with decreased risk of low birth weight, preterm delivery and early neonatal death.

Abstract 316 words.

Key Words: STDs, HIV, birthweight, preterm, neonatal mortality, perinatal HIV transmission.

Introduction

Sexually transmitted diseases (STDs) and other genital tract infections during pregnancy have serious sequelae, including increased rates of pregnancy loss and neonatal mortality,[1] chorioamnionitis, low birth weight, preterm birth and premature rupture of membranes,[2] congenital infections[3–5] and maternal upper genital tract infections.[6] STDs and chorioamnionitis have been associated with increased rates of maternal-to-infant HIV transmission,[7] and STDs have been implicated as risk factors for HIV acquisition in adults.[8] Thus, the control of STDs is a public health priority, particularly in developing countries where rates of these infections are high, and where access to adequate antenatal and intrapartum care is limited.[9] We conducted a community randomized trial of STD Control for HIV Prevention in rural Rakai district, southwestern Uganda.[10] Consenting pregnant women identified in this trial were enrolled into a nested maternal-infant follow up study which afforded an opportunity to assess the efficacy of STD control during pregnancy on a variety of maternal and fetal/infant outcomes. The results with respect to adult STD and HIV effects have been published,[10] and we report here the effects of STD control during pregnancy on maternal and infant health.

Methods

Trial design, population, intervention and data collection

The Rakai STD Control for HIV Prevention Trial was conducted between November 1994 and October 1998, and the design has been reported elsewhere.[10,11] All consenting adults aged 15-59 years, resident in 56 dispersed rural communities located on secondary roads were enrolled. Communities were aggregated into 10 clusters, and five clusters were randomly allocated to a STD intervention arm and five clusters to a control arm. Clusters were initially surveyed in random order to obtain baseline data and then re-surveyed at 10 month intervals. Participants were blinded with respect to study arm. Non-pregnant consenting intervention arm subjects received oral, single dose, directly observed therapy with azithromycin 1000 mg and ciprofloxacin 250 mg on one day, followed by metronidazole 2 grams on the next day. Ciprofloxacin is an FDA category

C drug which is contraindicated during pregnancy, was replaced by cefixime 400 mg (FDA category B) in intervention arm pregnant women. Intervention arm subjects with positive syphilis serology also received intramuscular Benzathine penicillin (2.4 million IU) provided in the home. This "mass" treatment covered symptomatic and asymptomatic infections, as well as partners in sexual networks within the study communities. The regimen is effective against syphilis, gonorrhea, chlamydia, chancroid, trichomonas and bacterial vaginosis,[10] and against a variety of non-STD pathogens, including group B *Streptococcus*.[12]

Non-pregnant control arm subjects received an iron/folate and low dose multivitamin regimen on the first day, followed by mebendazole on the next day. Mebendazole is a FDA category C drug and was withheld from control arm pregnant women. Control arm subjects found to have positive syphilis serology were offered their results in confidence and advised to obtain penicillin from the nearest government clinic. Particular efforts were made to inform all pregnant control women with serologic syphilis and to refer them for free treatment, and the Project provided government clinics with penicillin stocks to ensure availability. Control arm subjects with symptoms suggestive of STDs were provided with treatment at no cost in Project mobile clinics at the time of the survey, using Uganda Ministry of Health syndromic management regimens.

Because women were enrolled at varying durations of gestation there was no fixed timing of treatment administration during pregnancy in either study arm. All subjects, in both arms, who experienced STD symptoms between survey visits were advised to seek treatment in government clinics, and all participants received health education regarding STDs and HIV, condom promotion with access to free supplies, and confidential HIV testing and counseling, provided free of charge by Project.

Treatment and data collection occurred in the home at surveys conducted every ten months. Prior to each survey a census enumerated household residents and recorded births and deaths. At each survey, subjects were interviewed to determine their sociodemographic and behavioral characteristics, and health status. Subjects were asked to provide 5 ml venous blood and 10 ml of

urine for STD diagnoses, and women were asked to provide self-collected vaginal swabs for diagnosis of vaginal infections.

Because treatment regimens were modified for pregnant women in both study arms, we screened women of reproductive age (15-49 years) for pregnancy by interview and by hCG testing of urine (Card® Q.S.® hCG, Pacific Biotech Inc. San Diego, CA, USA). Women found to be pregnant were visited by a study midwife to obtain informed consent for enrollment in the maternal-infant follow up study. The consent statement described study requirements, benefits and risks of participation, and the right to refuse participation without loss of benefits. Consenting mothers were interviewed to ascertain their prior and current pregnancy history, including the date of the last normal menstrual period (LMP), and were questioned regarding abnormal vaginal bleeding, edema, vaginal discharge, dysuria and genital ulcer disease. At the enrollment visit, midwives provided basic antenatal care including abdominal examination, blood pressure, and testing of urine for proteinuria and glucose, but a pelvic exam was not routinely performed during home visits.

Mothers were asked to nominate a person (e.g., husband, relative or community health worker) to inform Project midwife as soon as possible after delivery. Women were also asked to note the timing of membrane rupture relative to the onset of labor pains, and the duration of membrane rupture prior to delivery. Project midwives visited the mother after notification of a delivery or other outcome of pregnancy (e.g. spontaneous abortion or stillbirth). Mothers were interviewed to ascertain use of antenatal care, symptoms of pregnancy complications (vaginal bleeding/spotting, edema) and the course of delivery, including the duration of labor, duration of membrane rupture, and rupture of membranes before onset of labor pains (i.e., premature rupture of membranes). The midwife performed an examination to diagnose postpartum upper genital tract infection (fever, purulent lochia , lower abdominal tenderness/guarding, cervical motion tenderness).[13] Women with suspected postpartum infection were treated with doxycycline and IM ceftriaxone in the home, in both study arms. Maternal blood, urine and vaginal swabs were obtained for STD diagnoses. In HIV-positive mothers and a sample of HIV-negative mothers with serologic syphilis, blood was also obtained for CD4 counts. Although syphilis treatment during

pregnancy was provided in both arms, mothers with persistent positive serology postpartum were given IM Benzathine penicillin (2.4 million IU) as an additional precaution.

Infants were examined for signs of congenital infection (ophthalmia neonatorum and congenital syphilis), or other morbidity. Infant head circumference was measured at the widest parietal diameter and chest circumference was measured at the level of the nipples at full inspiration. These parameters were used as surrogate indices for low birth weight.[14,15] The infant was weighed naked using a SECA scale accurate to 100 gm, which was calibrated to zero prior to each measurement. Anthropometric measurements were performed twice, and in cases of discrepancies, a third measurement was performed. The average of the first two measurements, or the two closest measurements if a third was taken, were used in analyses. Length was not measured because of the distress caused to the infant by use of a measuring board. To assess gestational maturity, we used the New Ballard Score which consists of six physical and six neuromuscular signs of maturity,[16] and has been validated against ultrasound, other maturity scores and LMP estimates of gestational age.

At the postpartum visit, a swab was taken from the infant's eyes for detection of gonorrheal and chlamydial ophthalmia by ligase chain reaction (LCR), and all infants received prophylactic erythromycin (0.5%) eye ointment after collection of the ocular sample. Infants with suspected gonorrheal ophthalmia were given a single intramuscular injection of ceftriaxone 125 mg.[4] Infant blood samples were obtained by heel sticks from infants born to both HIV-positive and HIV-negative mothers to avoid stigmatization. If mothers had been seropositive for syphilis during pregnancy or postpartum, their asymptomatic infants received IM Benzathine penicillin G 150,000 IU. Suspected congenital syphilis was detected using the Center for Disease Control (CDC) criteria for presumptive diagnosis,[3] and suspected cases were treated with IM Benzathine Penicillin G 150,000 IU and Procaine Penicillin G 150,000 IU, and referred for follow up treatment to the nearest government clinic.[3]

Infants of HIV-positive mothers, as well as infants born to mothers with positive syphilis serology were also followed up at 4-6 weeks of life for repeat blood samples to determine

mother-to-child HIV transmission by PCR and for repeat syphilis serology. To avoid stigmatization, blood was also collected on a subsample of infants born to uninfected mothers. No antiretroviral therapy was available and the study was conducted prior to the results from trials of short-course AZT or nevirapine.[17-19]

All infants were subsequently followed to ascertain morbidity and mortality. Deaths of mothers were identified during household censuses and interviews conducted with relatives to determine whether the death occurred during pregnancy or within approximately 42 days of delivery (i.e., probable maternal deaths). Infant deaths were reported by the mother who was interviewed to ascertain probable cause of death using "verbal autopsy" methods.[20] In cases where the mother and infant had died, the nearest relative was interviewed.

The trial was reviewed and approved by the AIDS Research Subcommittee of the Uganda National Council for Science and Technology, and by institutional review boards at the Uganda Virus Research Institute, Johns Hopkins and Columbia Universities, and the Office for Protection from Research Risk of the National Institutes of Health. The study was monitored by an NIH appointed, independent, Data Safety and Monitoring Board (DSMB). In January, 1998 the DSMB determined that the main STD Control Study would not achieve the primary end point of reducing HIV incidence and, based on a predetermined stopping rule, the trial was discontinued.[10] Subjects were unblinded with respect to study randomization arm, but home visits were continued to provide participants with information on the study results, and to administer the STD treatment regimen in both intervention and control arms, because of the health benefits established prior to termination of the randomized trial. The analyses presented here report findings on mothers who were enrolled prior to time of unblinding and trial termination.

Laboratory tests.

Maternal venous blood was collected for serologic diagnosis of HIV-1 using two different EIA assays (Vironostika HIV-1, Organon Teknika, Charlotte, NC and Cambridge Biotech, Worcester MA), with Western blot (WB) confirmation of discordant EIA tests (HIV-1 WB, Bio-

Merieux-Vitek, St Louis, MS). Syphilis diagnosis used the Toluidine Red Unheated Serum Test (TRUST™ , New Horizons, Columbia MD) as a nontreponemal screen and measure of titer, and TRUST-positive samples were confirmed by treponemal tests (TPHA Sera-Tek, Fujibero, Tokyo, Japan, or FTA-ABS IFA Test System, Zeus Scientific, Raritan, NJ). Maternal urine samples and infant ocular swabs were tested by ligase chain reaction for *N. gonorrhoeae* and *C. trachomatis* (LCx Probe System™ , Abbott Laboratories, Abbot Park IL). Due to assay costs, LCR was only done on a random sample of maternal urines and infant ocular swabs. For mothers who declined to provide a blood sample or who provided insufficient blood, maternal urine was also tested for HIV-1 using EIA (Calypte HIV-1 Urine EIA, Calypte Biomedical, Alameda, CA) with WB confirmation,. Self-collected vaginal swabs were tested for *T. vaginalis* using InPouch™ culture (BioMed Diagnostics, San Jose, CA) and for morphologic diagnosis of bacterial vaginosis (BV) using Gram stained slides.[21]

CD4 lymphocyte count was determined by TRAx® CD4 Test Kit (T Cell Sciences Inc. Needham, MA, USA), a micro titre sandwich enzyme-immunoassay which provides a quantitative measurement of CD4 T lymphocytes equivalents per µL of blood. The TRAx® CD4 test is suitable for a field laboratory and has been validated against flow cytometry.[22] Infant HIV-1 infection was detected by reverse transcriptase polymerase chain reaction (RT-PCR) using the Amplicor HIV-1 Monitor 1.5 Assay (Roche Molecular Systems, Branchburg, NJ)[23] on dried blood spots and sera. The assay is sensitive for HIV-1 subtypes A and D found in Uganda. Infant PCR results are available for 351 babies born to HIV-infected mothers; 117 (33.3%) provided samples only during the first 0-6 days of life, and 234 (66.6%) provided samples between one week and 6 weeks postpartum.

Trial End Points and Statistical Analyses

The trial was designed to assess multiple maternal-infant study endpoints. a) Survival, including pregnancy loss (spontaneous abortion/stillbirths), early neonatal death (within the first 7 days of life), and maternal mortality. b) Maternal STDs at the time of the postpartum visit

diagnosed by laboratory assays, and maternal upper genital tract infections diagnosed clinically. c) Infant ophthalmic infection detected by LCR for gonorrhea or chlamydia. d) Assessment of membrane rupture included prolonged membrane rupture prior to delivery ($ 4 hours), and premature rupture of membranes (PROM), before onset of labor pains. e) Maternal HIV acquisition during pregnancy was defined as seroconversion in women who were HIV-negative at time of enrollment during pregnancy and who were found to be HIV-positive at time of postpartum visit. f) HIV acquisition after delivery was assessed among women who were HIV-negative at the postpartum visit, but who had seroconverted by the next follow up blood draw. g) Maternal-to-infant HIV transmission was determined in infants born to HIV-positive mothers by HIV RT-PCR at the 1-6 week blood draw or, if an infant sample was not available between 1-6 weeks, the postpartum 0-6 blood draw was used. h) Maternal HIV viral load and CD4 count at the postpartum visit were measured as continuous variables. i) Infant anthropometric and gestational maturity outcomes were determined for singleton births observed within the first two weeks of life. For infants observed 24 hours or more after birth, anthropometric measurements were extrapolated to estimate indices on the day of birth using cubic spline regression models.[24] Mean and median birth weights were determined. Low birth weight (<2,500 grams), was determined by cubic spline extrapolated infant weights, or by two surrogate measures: a chest circumference less than 30 centimeters[14] and by an algorithm of chest and head circumference (head circumference #31 cms, or head circumference >31 cms with a chest circumference <30 cms)[15] which are sensitive and specific proxy measures of low birth weight in African populations.[14,15] Gestational age estimates were based on Ballard scores[16] extrapolated by cubic spline models to day of birth,[24] and by date of LMP to date of birth. Preterm birth was defined as a gestational age # 36 weeks, and preterm low birth weight was defined as a birth weight <2,500 grams with a gestational maturity # 36 weeks.

All study endpoints were evaluated by an intent-to-treat analysis with adjustment for cluster randomization. Univariate and stratified analyses were used to assess factors associated with trial outcomes. Potential confounding factors were included in multivariate analyses if statistically significant in univariate analyses ($p < 0.05$), or if the unadjusted rate ratio was $ 2. Variables found to differ between intervention and control arm at enrollment were also

considered. Multivariate logistic regression was used to estimate adjusted risks for prevalent outcomes such as maternal or infant infections, infant anthropometry and gestational maturity, and mother-to-child HIV transmission.[25] Maternal HIV incidence was estimated per 100 person years (py), assuming that infection occurred at the mid-interval between successive HIV tests and the adjusted risk of HIV acquisition was assessed by multivariate Poisson regression.[26] Cluster adjusted rate ratios (RR) of outcome events in the intervention relative to control arms were calculated as geometric means of cluster rate ratios based on observed to expected events. Ninety-five percent confidence intervals (95%CI) were estimated from *t* intervals of log-transformed rates with equal weighting per cluster, by comparison of observed to expected events in each cluster,[27] using logistic or Poisson multiple regression, as appropriate. For HIV endpoints, clusters were paired by baseline HIV prevalence and cluster-pairs were used in analysis. Covariates included in the models differed by end point of interest and are listed as foot notes to the relevant tables.

Results

Trial Profile

The trial profile is given in Figure 1. We identified 2,339 pregnant women in the intervention and 2,330 pregnant women in the control arm prior to the trial termination. Among these women, 198 in the intervention arm and 279 in the control arm were absent when the midwives attempted to enroll them. There were 2,141 intervention arm mothers contacted, of whom 2,072 consented to the study (96.8%), and in the control arm, 2,051 mothers were contacted, of whom 1,964 consented (95.8%). Among consenting subjects, 94.7% in the intervention arm (1,962/2,072) and 97.0% in the control arm (1,905/1,964) accepted treatment. Postpartum follow up was achieved for 94.5% of enrolled mothers in the intervention arm (1,958/2,072) and 92.7% in the control arm (1,820/1,964). Losses to follow up were due to refusal (0.8% in the intervention and 1.7% in the control), maternal deaths (0.5% in intervention and 0.4% in the control), and maternal absences among women who delivered in their parental villages outside the study catchment area (4.2% in the intervention, 5.2% in the control arm). Pregnancy loss rates between enrollment and postpartum visit were 5.1% in the intervention and 4.9% in the control arm.

In the intervention arm, the 1,958 mothers followed up postpartum gave birth to 1,888 live born infants of whom 48 died (2.5%) prior to the postpartum visit, and 1,840 surviving infants were seen postpartum. In the control arm, the 1,820 mothers gave birth to 1,755 live born infants, of whom 51 died (2.9%) prior to postpartum follow up, and 1,704 surviving infants were seen postpartum. The effect of the intervention on birthweight and gestational maturity among these surviving infants was assessed for singleton infants observed during the first two weeks of life. In the intervention arm, there were 1,791 singleton births of whom 1,478 (82.5%) had anthropometric measures within 14 days of life, and in the control arm there were 1,659 singleton infants, of whom 1,266 (76.3%) had anthropometry during the first two weeks of life. The lower proportion of control arm infants with anthropometry within 14 days of birth was due to delays in follow up early in the trial as a consequence of resource constraints, which differentially affected control clusters.

There were 430 HIV-infected mothers, 251 in the intervention and 176 in the control arm. Infant blood samples were obtained for 351 babies born to the 430 HIV-positive mothers (81.6%); 207/251 in the intervention arm (81.5%) and 144/176 in the control arm (81.8%).

Comparability of Intervention and Control Arm Mothers at Enrollment During Pregnancy and at Postpartum follow up.

Table 1 shows the characteristics of consenting mothers at time of enrollment during pregnancy. Intervention and control mothers were comparable with respect to age. More mothers in the intervention than the control arm had never married or been in a stable consensual union (12.4% and 7.6%, respectively), and conversely, a lower proportion of intervention mothers were currently married compared with the controls (81.5% and 86.5%, respectively, $p < 0.001$). The intervention arm mothers more frequently had formal education than the control mothers (91.7% vs 87.4%, $p < 0.001$). However, the two groups were similar with regard to parity, history of spontaneous abortion or stillbirth in previous pregnancies, trimester of pregnancy at time of enrollment and a history of vaginal bleeding, discharge or genital ulcer disease (GUD).

Approximately half of the mothers had received some antenatal care for the current pregnancy, prior to their enrollment into the study.

Table 2 shows the history of pregnancy, labor and delivery reported at the postpartum contact. A similar high proportion of intervention and control mothers reported receiving antenatal care (from non-Project services) during their pregnancy, and the two groups were comparable with respect to a history of vaginal discharge, dysuria, GUD, malaria and edema during pregnancy. There were no significant differences between randomization arms in the number of reported sexual partners during pregnancy, or intercourse during the last month of gestation. The timing of the postpartum follow up differed significantly between the two arms. This was due to resource constraints which delayed postpartum follow up visits disproportionately in control arm clusters which, by random assignment, were over-represented among the communities surveyed earlier in the investigation. Receipt of HIV test results and counseling was reported by 47.0% of intervention arm and 51.3% of control arm women.

Pregnancy Loss, Maternal and Infant Mortality, and Complications of Delivery

Pregnancy loss, maternal and infant mortality, and delivery complications are shown in Table 3. There were no statistically significant differences in rates of pregnancy loss between the intervention and control arm, but early neonatal mortality was significantly lower in the intervention arm (25.4 per 1000 births) than the control (29.1 per 1000 live births; cluster adjusted OR= 0.83, 95%CI 0.71-0.97). The verbal autopsy information was not adequate to assess cause of death, so cause-specific mortality could not be determined. Maternal mortality was higher in the intervention than in the control arm (532 versus 357 per 100,000 births, respectively), but these differences were based on small numbers of maternal deaths, and were not statistically significant. Seven of the 18 maternal deaths occurred among women who were known to be HIV-infected, 4 in the intervention arm and 3 in the control. Maternal mortality among women known to be HIV-positive was 7 out of 415 mothers, (1,687 per 100,000 births), compared with 11 maternal deaths out of 3,325 HIV-negative women (331 per 100,000 births), and the rate ratio of maternal mortality in HIV-positive relative to HIV-negative women was 5.10 (95%CI 1.99-13.08).

Maternal and Infant STD and HIV Infections

Maternal and infant STD infections at the postpartum visit are given in table 4. There were no significant differences between randomization arms in serological syphilis at the postpartum visit, because, for ethical reasons, serological syphilis was aggressively managed in both arms. The prevalence of vaginal infections was significantly lower in the intervention arm compared to control arm; for *T. vaginalis* the cluster adjusted RR was 0.28 (95%CI 0.18-0.49), and for BV the RR was 0.78 (95%CI 0.69-0.87). Due to assay costs, LCR detection of *N. gonorrhoeae* and *C. trachomatis* were done on random samples of 1,503 intervention arm and 1,394 control arm mothers (i.e., 76.8% of intervention arm and 76.6% of control arm mothers with postpartum follow up). The point prevalence of these two cervical infections was lower in the intervention arm subjects (RR = 0.43, 95%CI 0.27-0.68). The prevalence of clinically diagnosed upper genital tract infection was lower in the intervention than in the control arm, but this difference was not statistically significant (RR = 0.76, 95%CI 0.53-1.09). Infant ocular gonorrhea and chlamydia infections were lower in the intervention arm compared with the control arm, and the rate ratio for the two infections combined was 0.37, 95%CI 0.20-0.70.

Table 5 shows maternal HIV prevalence, maternal HIV acquisition and maternal-to-child HIV transmission. The prevalence of HIV after delivery was not significantly different between intervention and control mothers. The overall HIV incidence rate during pregnancy and following delivery was 3.4 per 100 py in the intervention and 2.3 per 100 py in the control arms (RR = 1.44, 95%CI 0.64-3.25). Maternal HIV acquisition was determined during pregnancy (i.e., between the enrollment and postpartum visit) in 1,022 HIV-negative women in the intervention arm and 1,008 HIV-negative women in the control arm. HIV incidence during pregnancy was 4.0 per 100 py in the intervention and 2.5 per 100 py in the control arm, which was not statistically significant ($p = 0.63$). HIV seroconversion following delivery (i.e., between the postpartum visit and the subsequent study survey), was determined in 703 HIV-negative intervention arm mothers and in 519 HIV-negative control arm mothers; the incidence of HIV was 2.7 per 100 py in the intervention and 2.1 per 100 py in the control arms mothers.

The overall mother-to-child HIV transmission rates were 18.4% in the intervention and 20.8% in the control, which was not statistically significant (Table 5). Among infants with blood samples drawn within the first seven days of life, the transmission rate was 17.2% (13/75) in the intervention arm and 17.1% (7/41) in the control (cluster pair adjusted RR = 0.74, 95%CI 0.08-6.52). Among infants with samples at more than one week of age, the transmission rate was 18.92% (25/132) in the intervention arm, and 22.6% (23/102) in the control arm. This difference was not statistically significant (cluster pair adjusted RR = 0.92, 95%CI 0.29-2.91). The mean and median maternal HIV viral loads were 44,455 and 14,180 copies per µL, respectively, in the intervention arm, and 58,168 and 14,786 copies per µL in the control. The $\log_{10}$ mean viral loads were 4.0819 (se = 0.0593) in the intervention, and 4.1347 (se = 0.07256) in the control arm. This difference is not statistically significant. The mean CD4 counts were similar in the intervention and control arms (691.4 and 668.9 per µL, respectively).

Birthweight and Gestational Maturity

Anthropometry and gestational age were analyzed for singleton infants observed during the first two weeks of life (Table 6). For those infants seen within the first 14 days of life the mean delay between birth and observation was 3.2 days (se = 0.07) in the intervention arm, and 3.7 days (se = 0.08) in the control arm (p = 0.01). The median delays were 2.5 and 3.0 days, respectively. Measures were extrapolated to day of birth by cubic spline models so as to adjust for differences in the day of observation postpartum and the variation in measures by age of the infant.[24] The estimated prevalence of low birth weight (< 2,500 grams) based on extrapolated weight did not differ between the intervention and control arms. The estimated mean birth weight was similar in both arms; 3,081.9 grams ± SD 567.8 for the intervention arm infants, and 3,081.0 ± SD 517.9 for the control arm infants. However, the proportion of babies with a chest circumference <30 cms (a surrogate for low birth weight),[14] was significantly lower in the intervention than the control arm (RR = 0.68, 95%CI 0.53-0.86), as was the algorithm for low birth weight based on combined head and chest circumference[15] (RR = 0.76, 95%CI 0.59-0.99). We examined the timing of treatment

during pregnancy associated with the prevalence of low birth weight as indicated by a chest circumference <30 cms. Among women enrolled during the first half of pregnancy, the prevalence of low birth weight was 9.8% (52/529) in the intervention arm and 12.3% (55/447) in the control (RR = 0.69, 95%CI 0.40-1.19), and among women enrolled in the second half of pregnancy, the prevalence of low birth weight was 8.7% (74/851) in the intervention arm and 10.1% (74/733) in the control, which was statistically significant (RR = 0.72, 95%CI 0.54-0.95). The proportion of infants with preterm delivery (#36 weeks) was lower in the intervention compared with the control arm and this difference was of borderline statistical significance (RR=0.77, 95%CI 0.56-1.05, p = 0.06), but there was no significant difference in the proportion of preterm low birth weight infants.

Discussion

This community randomized trial found significant reductions in maternal cervical and vaginal infections, and infant ophthalmic infections following mass treatment with a broad spectrum of antibiotics during pregnancy (Table 4). Despite these reductions in infection rates, the intervention did not affect maternal HIV acquisition or mother-to-child transmission of HIV (Table 5). Early neonatal mortality was significantly lower in the intervention arm (Table 3), and there were significant reductions in the proportions of infants with low birth weight (estimated from chest and head circumference), as well as a reduction in preterm delivery which was of borderline significance (Table 6). This suggests that the improved infant outcomes may be due to the lower prevalence of maternal STD infections in the intervention arm population.

There are public health implications of these findings, both for control of infection during pregnancy and for HIV prevention. Clearly, STD infections during pregnancy are a major public health problem in this rural population, as in other sub-Saharan African settings, and previous efforts to control these infections by serological screening for syphilis or syndromic diagnosis have not been notably successful.[9,28] In the present trial, screening for syphilis during pregnancy

and provision of accessible penicillin treatment was, for ethical reasons, provided to pregnant women in both arms and the prevalence of serologic syphilis declined from approximately 10% at baseline[10] to around 3.0% following delivery in both study arms (Table 4). The high prevalence of serologic syphilis among pregnant women observed in this and other African studies,[3,9] clearly indicates the need for effective syphilis management in pregnancy. The intensive mass treatment with oral antibiotics in the intervention arm was effective in reducing vaginal and cervical infections in the mothers, infant ophthalmia due to gonorrhea and chlamydia, and early neonatal mortality. The reduction in early neonatal mortality may reflect the impact of the intervention on maternal-infant STDs or an effect on other bacterial infections such as group B *Streptoccocus*.[12] We could not diagnose deaths due to neonatal sepsis on the basis of the verbal autopsy reports, but a randomized trial in Malawi, which controlled neonatal infection by chlorhexadine cleansing of the birth canal during labor, found significant reductions in neonatal sepsis morbidity and mortality.[29] This suggests that a reduction in maternal genital tract infections is a plausible explanation for the reduction in neonatal mortality in the present study. The effects of the intervention on the prevalence of low birth weight or preterm delivery were modest (Table 6), but these outcomes may have made some contribution to the lowered early neonatal mortality.

Our findings that trichomonas and BV prevalence, low birth weight and preterm delivery were lower in the intervention compared with the control arm, is consistent with observational studies.[2] Some, but not all, randomized trials of treatment for BV during pregnancy have shown reductions in preterm birth, particularly among high risk women.[2] Thus, it is reasonable to conclude that treatment of these highly prevalent vaginal infections during pregnancy is likely to be beneficial. Since metronidazole is inexpensive (approximately US 25 cents per 2 gram dose), and there is no evidence of toxicity during pregnancy,[30] consideration should be given to more widespread use of this drug, especially in populations with high rates of BV and trichomoniasis, as in Rakai or other sub-Saharan African settings.

With regard to the implications of our findings for HIV control during pregnancy, it is disappointing that we failed to observe an effect on maternal HIV acquisition or maternal-to-child

HIV transmission, despite significant reductions in maternal cervical and vaginal infections. However, the study had limited power to detect effects on maternal-to-child transmission. The absence of an effect on maternal HIV acquisition during pregnancy or after delivery is consistent with our failure to detect an impact on adult HIV incidence in the main Rakai STD control trial,[10] but is at variance with the findings from one other randomized trial in Mwanza, Tanzania, which observed significantly lower adult HIV incidence following improved syndromic management of STDs.[31] The Mwanza study did not assess HIV incidence in pregnant women or maternal-to-child HIV transmission, and found no effect of syndromic management on STD prevalence during pregnancy.[28,31] In Rakai, the incidence of maternal HIV acquisition during pregnancy was higher than during the postpartum period or in the general population of women of reproductive age.[10] Studies in Malawi have also observed high rates of seroconversions during pregnancy.[32] These findings suggests that pregnant women may be particularly vulnerable to HIV acquisition, perhaps as a consequence of hormonal factors or gestational changes in the genital tract, and there clearly is a need to promote condom use and safe sex behaviors to avoid HIV infection during pregnancy.

On the basis of observational studies, it has been suggested that genital tract infections and chorioamnionitis act as cofactors for perinatal HIV transmission, and that treatment of these conditions may reduce mother-to-child HIV transmission.[7] However, we did not observe an effect of the intervention on maternal-to-infant HIV transmission although rates of cervical and vaginal infections were reduced by the intervention. A trial of chlorhexadine birth canal washing during labor in Malawi also failed to demonstrate a reduction of perinatal HIV transmission (except in a subgroup of mothers with prolonged rupture of membranes > 4 hours),[33] despite significant reductions in maternal and neonatal sepsis.[29] The Malawian and Rakai trials do not provide direct support for the efficacy of treatment of chorioamnionitis on maternalto-child HIV transmission, but further trials of more targeted interventions are warranted. The observed rate of mother-to-child HIV transmission of 19.4% determined in this rural population largely reflects *in utero* and perinatal infections. These rates are comparable to those reported in some African studies,[34,35] but are lower than the rates of vertical transmission in other, predominantly urban, African populations.[17,19] This variation in the rates of mother-to-child transmission between study sites may reflect differences in urban/rural populations, variation in the timing of infant blood sampling, or

the sensitivity of the PCR assays used. As noted previously, short-course zidovudine and nevirapine therapy were not available at the time of this trial.[17-19]

There are several limitations which affect the interpretation of this trial. First, because the mother-infant study was nested within the larger STD Control trial, pregnant women were enrolled at varying gestations, and the intervention could not be provided on a fixed schedule during pregnancy. This is unlikely to have biased the comparison between randomization arms because the trimester of enrollment was similar in the intervention and control subjects (Table 1). Nevertheless, the variable timing of the intervention during pregnancy may have reduced the power to detect the efficacy of the STD intervention, if this was time dependent. A second limitation is that, for ethical reasons, we actively screened and treated maternal serologic syphilis in both study arms, so the impact of syphilis treatment could not be evaluated. Finally, because the majority of deliveries occurred in the home and there were unavoidable delays in assessing new born infants, the anthropometry and gestational age measurements could not be obtained on the day of birth. We extrapolated these latter outcomes to estimate parameters on the day of birth by cubic spline models, but such extrapolation could lead to imprecision and misclassification, particularly of birth weight, which varies substantially with infant age.[24] Surrogate measures based on chest and head circumference are more stable during the first two weeks of life, and we observed significant treatment associated reductions in the prevalence of these stable proxy indicators.

In conclusion, the treatment of STDs and genital tract infections during pregnancy improved maternal and infant health, and operations research is needed to determine the optimal strategy for programmatic interventions, particularly targeting common vaginal infections such as trichomonas and BV. However, the treatment of these lower genital tract infections did not reduce the rates of maternal HIV acquisition or maternal-to-infant HIV transmission, so these interventions may not be appropriate for HIV prevention in a mature, generalized HIV epidemic setting.

Trial Profile

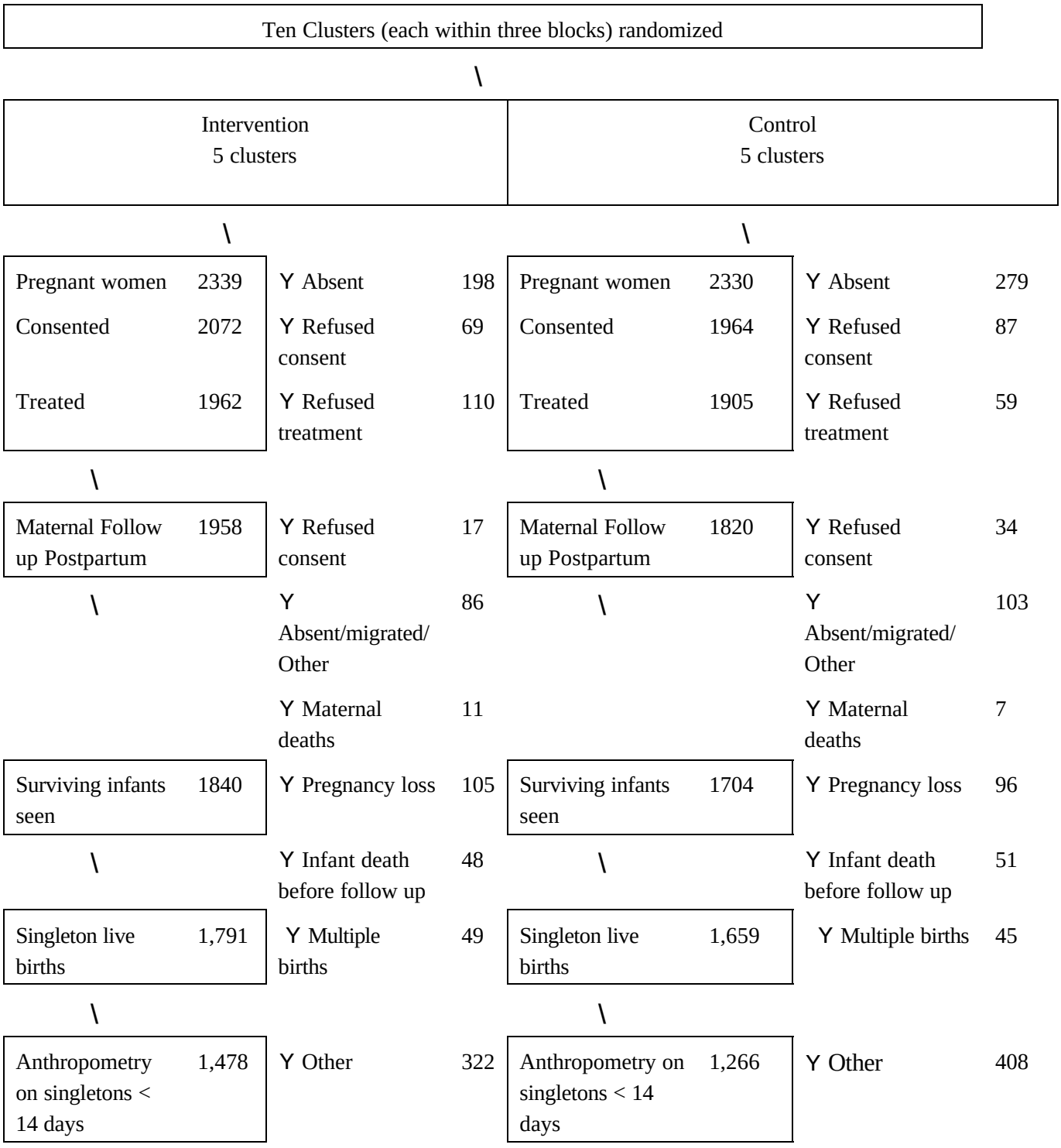

Table 1. Sociodemographic and obstetric characteristics at enrollment, obtained at enrollment.

Characteristics Prior to Pregnancy		Intervention (N = 2,063)		Control (N = 1,957)	
Age		Number	Percent	Number	Percent
15-19		535	25.9	476	24.3
20-24		715	34.7	717	36.6
25-29		403	19.5	390	19.9
30-39		371	18.0	338	17.3
40-49		36	1.8	37	1.9
Marriage:	Never married	257	12.4	149	7.6***
	Currently married	1681	81.5	1693	86.5
	Separated/divorced/widowed	125	6.1	115	5.9
Education:	No education	171	8.3	246	12.6***
	Primary education	1430	69.3	1358	69.3
	Secondary and above	462	22.4	353	18.0
Parity:	Nulliparous	417	20.2	365	18.7
	Para 1	403	19.5	379	19.4
	Para 2-3	573	27.8	578	29.5
	Para 4-5	367	17.8	328	16.8
	Para 6+	303	14.7	307	15.7
Prior miscarriage/previous pregnancy		323/1686	19.2	285/1635	17.4
Prior stillbirth/previous pregnancy		132/1686	7.8	108/1635	6.6
1st trimester at time of enrollment		802	38.9	741	37.9
2nd trimester at time of enrollment		678	32.8	610	31.2
3rd trimester at time of enrollment		531	25.7	545	27.9
Unknown		52	2.5	61	3.1
Illnesses:	Vaginal bleeding	59	2.9	69	3.5
	Vaginal discharge	410	19.9	423	21.6
	Genital ulcer	137	6.6	107	5.5
Any antenatal care		1035	50.2	953	47.9

*** $?^2$ $p<0.001$

Table 2. History of pregnancy and delivery obtained at postpartum visit.

History During Pregnancy	Intervention		Control	
	Number	Percent	Number	Percent
Any antenatal Care	1782/1958	91.0	1605/1818	88.3
Symptoms of illness during pregnancy				
Vaginal discharge	335/1958	17.1	309/1816	17.0
Dysuria	120/1957	6.1	139/1815	7.7
GUD	58/1958	3.0	62/1816	3.4
Malaria	303/1958	15.5	324/1816	17.8
Edema	89/1958	4.6	112/1816	6.2
Number of sex partners during pregnancy				
None	12/1952	0.6	8/1804	0.4
1 partner	1910/1952	97.9	1772/1804	98.2
2+ partners	30/1952	1.5	23/1804	1.3
Intercourse during last month of pregnancy	1016/1952	52.1	912/1800	50.7
Day of postpartum at assessment	n = 1958		n = 1818	
0 (day of birth)	34	1.7	16	0.9
1	292	14.9	217	11.9
2	454	23.2	335	18.4
3	290	14.8	244	13.4
4	165	8.4	147	8.1
5	99	5.1	97	5.3
6	56	2.9	68	3.7
7-13	165	8.4	190	10.5
14+	403	20.6	504	27.7***
Received HIV Test Results and Counseling	823/1752	47.0	861/1677	51.3

*** $?^2$ $p<0.001$

Table 3. Pregnancy loss, maternal and infant mortality and labor/delivery

	Intervention Arm (N = 2,070)		Control Arm (N = 1,963)		Cluster Adjusted Rate Ratio (95% Confidence Intervals)
	Events/ Number at risk	Rate	Events/ Number at risk	Rate	
Spontaneous abortion[1] (%)	35/1993	1.8	46/1850	2.5	0.80 (0.23-2.82)
Stillbirths (%)[1]	70/1993	3.5	50/1850	2.7	1.25 (0.70-1.83)
Early Neonatal Deaths[2] (per 1000 live births)	48/1888	25.4	51/1754	29.1	0.83 (0.71-0.97)
Maternal deaths (per 100,000 women)[3]	11/2068	532	7/1963	357	1.47 (0.59-3.66)
Premature rupture of membranes (%)[4]	69/1842	3.8	64/1672	3.8	1.02 (0.62-1.67)
Prolonged rupture of membranes > 4 hours (%) [4]	26/1850	1.4	28/1678	1.7	0.74 (0.39-1.40)
Prolonged labor > 12 hours (%) [4]	548/1902	28.8	541/1747	31.0	0.93 (0.80-1.07)
Antepartum hemorrhage (%)	42/1950	2.2	51/1806	2.8	0.77 (0.47-1.26)

[1] Denominator for spontaneous abortions and stillbirths was pregnancies with postpartum follow up

[2] Denominator for early neonatal deaths was live births with follow up.

[3] Denominator for maternal deaths was all women known to be pregnant and whose survival status was ascertained either by direct follow up mothers known to be alive or to have died, including those who were absent or refused but whose survival status was known.

[4.] Denominator includes mothers who were able to report timing and duration of membrane rupture, and duration of labor.

Table 4. Maternal and infant STD infections by intervention and control arm

Maternal and Infant STDs	Intervention Arm		Control Arm		Cluster Adjusted Rate Ratio (95% Confidence Intervals)†
	Infections/ Number tested	Prevalence (%)	Infections/ Number tested	Prevalence (%)	
Prevalence of Maternal Infections					
Syphilis	57/1677	3.4	46/1376	3.3	1.18 (0.94-1.47)
Trichomonas	84/1779	4.7	248/1569	15.9	0.28 (0.18-0.49)
Bacterial Vaginosis	645/1779	36.3	764/1576	48.5	0.78 (0.69-0.87)
Gonorrhea	14/1503	0.9	24/1394	1.7	0.53 (0.27-1.05)
Chlamydia	16/1503	1.1	38/1394	2.7	0.34 (0.16-0.71)
Gonorrhea and/or Chlamydia	29/1503	1.9	60/1394	4.3	0.43 (0.27-0.68)
Upper genital tract infection	51/1950	2.6	63/1808	3.5	0.76 (0.53-1.09)
Prevalence of Infant Ocular Infections					
Gonorrhea	6/1022	0.6	17/1008	1.7	0.34 (0.19-0.62)
Chlamydia	6/1022	0.6	11/1008	1.1	0.44 (0.18-1.10)
Gonorrhea or Chlamydia	12/1022	1.2	28/1008	2.8	0.37 (0.20-0.70)

† Adjusted for age and number of sexual partners.